Glass transition in biomolecules and the liquid-liquid critical point of water

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Abstract

Using molecular dynamics simulations, we investigate the relation between the dynamic transitions of biomolecules (lysozyme and DNA) and the dynamic and thermodynamic properties of hydration water. We find that the dynamic transition of the macromolecules, sometimes called a "protein glass transition", occurs at the temperature of dynamic crossover in the diffusivity of hydration water, and also coincides with the maxima of the isobaric specific heat C_P and the temperature derivative of the orientational order parameter. We relate these findings to the hypothesis of a liquid-liquid critical point in water. Our simulations are consistent with the possibility that the protein glass transition results from crossing the Widom line, which is defined as the locus of correlation length maxima emanating from the hypothesized second critical point of water.

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Both experiments and computer simulation studies have shown that hydrated proteins undergo a "glass-like" transition near 200 K [1, 2, 3, 4, 5], above which proteins exhibit diffusive motion, and below which the proteins are trapped in harmonic modes. An important issue is to determine the effects of hydration water on this dynamical transition [6, 7, 8, 9, 10]. Experiments and computer simulations suggested that when a protein is solvated, the protein glass transition is strongly coupled to the solvent, leading to the question of whether the protein glass transition is directly related to a dynamic transition in the surrounding solvent [11].

It has been hypothesized that the deeply supercooled region of the phase diagram of liquid water may contain a first order phase transition between a high density liquid (HDL) and a low density liquid (LDL) [12]. This line of phase transition has a negative slope in the P-T phase diagram and terminates with a critical point, C_2 [12, 13, 14, 15], which is located at $T_{C2} = 200 \pm 20$ K and $P_{C2} = 250 \pm 100$ MPa [16]. Upon crossing the first order phase transition line above the critical pressure, the thermodynamic state functions change discontinuously. Below the critical pressure they rapidly but continuously change upon cooling.

Computer simulations of the TIP5P and ST2 models show that response functions such as isobaric specific heat C_P and isothermal compressibility have maxima as functions of temperature if the system is cooled isobarically at $P < P_{C2}$ [15]. The loci of these maxima asymptotically approach one another [17] as the critical point is approached, because response functions become expressible in terms of the correlation length which diverges at the critical point. The locus of the correlation length maxima is called the Widom line.

Experimental studies of supercritical water [18] indeed show that various response functions have sharp maxima in the region of the phase diagram above the liquid-vapor critical point C_1, but no direct experimental indication of a liquid-liquid critical point C_2 had been available due to unavoidable crystallization of bulk water. It was found that water remains unfrozen in hydrophilic nanopores for $T > 200$ K [19, 20]. Moreover when cooled at constant pressure for $P < P_{C2}$ the dynamics changes from non-Arrhenius to Arrhenius at $T = T_\times(P)$. The line $T_\times(P)$ is located in the range of temperatures between $200 - 230$ K and has a negative slope in the P-T phase diagram. Computer simulations suggest that this line may be associated with $T_W(P)$, the Widom line, near which the local dynamic characteristics must rapidly change from those resembling the properties of HDL at high temperature to those

of LDL at low temperature [15].

Here we explore the hypothesis [21] that the observed glass transition in biomolecules is related to the liquid-liquid phase transition using molecular dynamics (MD) simulations. Specifically, we study the dynamic and thermodynamic behavior of lysozyme and DNA in hydration TIP5P water, by means of the software package GROMACS [22] for (i) an orthorhombic form of hen egg-white lysozyme [23] and (ii) a Dickerson dodecamer DNA [24] at constant pressure $P = 1$ atm, several constant temperatures T, and constant number of water molecules N (NPT ensemble) in a simulation box with periodic boundary conditions. We first allow the system to equilibrate at constant temperature and pressure using the Berendsen method. This initial equilibration is followed by a long production run during which we calculate the dynamic and static properties. Equilibration times vary for different temperatures from a few ns for high temperatures to as much as 40 ns for low temperatures. The MD for DNA was performed using the Amber force field [25]. For lysozyme simulations, the system consists of a single protein in the native conformation solvated in $N = 1242$ TIP5P water molecules [14, 26, 27]. These simulation conditions correspond to a ratio of water mass to protein mass of 1.56. The DNA system consists of a single DNA helix with 24 nucleotides solvated in $N = 1488$ TIP5P water molecules, which corresponds to an experimental hydration level of 3.68.

The simulation results for the mean square fluctuations $< x^2 >$ of protein are shown in Fig. 1(a). We calculate the mean square fluctuations $< x^2 >$ of protein from the equilibrated configurations, first for each atom over 1 ns, and then averaged over the total number of atoms in the protein. We find that $< x^2 >$ [Fig. 1(a)] changes its functional form below $T_{\rm p} \approx 242 \pm 10$ K. Moreover, upon cooling, the diffusivity of hydration water exhibits a dynamic crossover from non-Arrhenius [28] to Arrhenius behavior at the crossover temperature $T_{\times} \approx 245 \pm 10$ K [Fig. 1(c)]. A similar temperature dependence of diffusivity of bulk TIP5P water was observed in Ref. [15]. The coincidence of $T_{\times}$ with $T_{\rm p}$ within the error bars indicates that the behavior of the protein is strongly coupled with the behavior of the surrounding solvent, in agreement with recent experiments [21]. Note that $T_{\times}$ is much higher than the glass transition temperature estimated for TIP5P as $T_g = 215$K [14]. Thus this crossover is not likely to be related to the glass transition in water. Here we will explore the possibility that instead it is related to a change in the properties of protein hydration water.

We next calculate C_P by numerical differentiation of the total enthalpy of the system

(protein and water) by fitting the simulation data for enthalpy with a fifth order polynomial, and then taking the derivative with respect to T. Figure 2(a) displays a maximum of $C_P(T)$ at $T_W \approx 250 \pm 10$ K for the case of lysozyme-water system [29]. The fact that $T_p \approx T_\times \approx T_W$ is evidence of the correlation between the changes in protein fluctuations [Fig. 1 (a)] and the hydration water thermodynamics [Fig. 2 (a)]. Thus our results are consistent with the possibility that the protein glass transition is related to the Widom line (and hence to the hypothesized liquid-liquid critical point). Crossing the Widom line corresponds to a continuous but rapid transition of the properties of water from those resembling the properties of a local HDL structure for $T > T_W(P)$ to those resembling the properties of a local LDL structure for $T < T_W(P)$ [15, 20]. A consequence is the expectation that the fluctuations of the protein residues in predominantly LDL-like water (more ordered and more rigid) just below the Widom line should be smaller than the fluctuations in predominantly HDL-like water (less ordered and less rigid) just above the Widom line.

To test this interpretation, we analyze the structure of hydration water on the two sides of the Widom line. Fig. 3(a) shows the oxygen-oxygen radial distribution function $g(r)$ on two sides of the Widom line for lysozyme hydration water. The first peak of $g(r)$ on the low temperature (T=230 K) side is sharper and the first minimum is shallower compared to the $g(r)$ on the high temperature (T=270 K, 300 K) side of the Widom line, suggesting that water is more structured on the low temperature side. Further, we calculate the structure factor $S(q)$ of lysozyme hydration water [Fig. 3(c)]. The first peak of the structure factor associated with the hydrogen bond is very sharp and pronounced, for $T < T_W(P)$, it is diminished and moves to larger wave vectors for $T > T_W(P)$, consistent with a LDL-like local structure for $T < T_W(P)$ and a HDL-like local structure for $T > T_W(P)$.

Previous simulations [10] and experiments [9] suggest a "glass-like" transition of DNA around temperature 230 K. Hence to test if the dynamic crossover depends on the solute, we performed a parallel study of the DNA Dickerson dodecamer [24]. We find that fluctuations [30] of the DNA molecule [Fig. 1(b)] change their behavior approximately at the same temperature as lysozyme, with $T \approx 247 \pm 10$ K. The dynamic crossover in the hydration water upon cooling from non-Arrhenius to Arrhenius behavior takes place at $T_\times \approx 250 \pm 10$ K [Fig. 1(d)]. For DNA hydration water system C_P has a maximum at $T \approx 250 \pm 12$ K, similar to the case of protein (see Fig. 2(b)) [31]. Fig. 3(b) and Fig. 3(d) show $g(r)$ and $S(q)$ for the DNA hydration water [32]. Further to describe the quantitative changes in structure

of hydration water, we calculate the local tetrahedral order parameter Q [33] for hydration water surrounding lysozyme and DNA. Figs. 3(e) and 3(f) show that the rate of increase of Q has a maximum at 245 ± 10 K for lysozyme and DNA hydration water respectively; the same temperatures where we find a crossover in the behavior of mean square fluctuations and a change in the behavior of the dynamics of hydration water.

The quantitative agreement of the results for DNA and lysozyme suggests that it is indeed the changes in the properties of hydration water that are responsible for the changes in dynamics of the protein and DNA biomolecules. Our results are in qualitative agreement with recent experiments on hydrated protein and DNA [34] which found the crossover in side-chain fluctuations at $T_{\mathrm{p}} \approx 225$ K .

Note Added in Proof: After this work was submitted, we learned of interesting parallel work on silicon, which also interprets structural change in $g(r)$ and $S(q)$ as crossing the Widom line [35].

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[29] The error bars in the enthalpy calculation do not exceed 1%. To assess the error bar on the position of the maximum we perform 1000 fittings of the data points by adding random Gaussian variables. The standard deviation of the Gaussian noise was taken to be the error bars in enthalpy.

[30] The $< x^2 >$ for DNA was calculated in the same fashion as for lysozyme.

[31] The values of C_P are different in each case with $C_P^{\mathrm{DNA}}(T_W) > C_P^{\mathrm{protein}}(T_W)$, probably because DNA hydration level (368%) is larger than lysozyme hydration level (156%) and the fact that

the specific heat of water is larger than protein and DNA.

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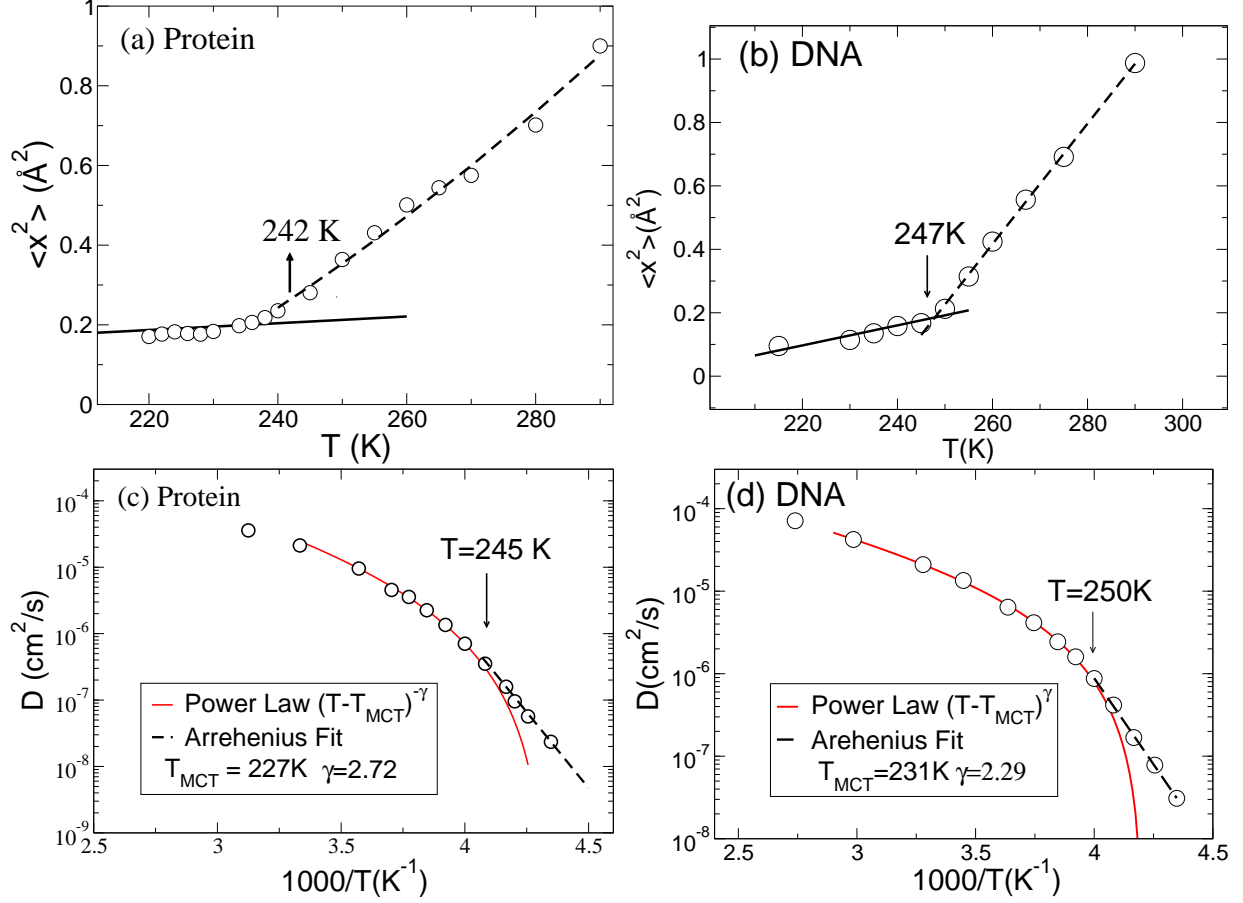[36] W. Götze, J. Phys.: Cond. Matt. **11**, A1 (1999).

FIG. 1: Mean square fluctuation of (a) lysozyme, and (b) DNA showing that there is a transition around $T_p \approx 242 \pm 10$ K for lysozyme and around $T_p \approx 247 \pm 10$ K for DNA. For very low T one would expect a linear increase of $< x^2 >$ with T, as a consequence of harmonic approximation for the motion of residues. At high T, the motion becomes non-harmonic and we fit the data by a polynomial. We determine the dynamic crossover temperature T_p from the crossing of the linear fit for low T and the polynomial fit for high T. We determine the error bars by changing the number of data points in the two fitting ranges. Diffusion constant of hydration water surrounding (c) lysozyme, and (d) DNA shows a dynamic transition from a power law behavior to an Arrhenius behavior at $T_\times \approx 245 \pm 10$ K for lysozyme and $T_\times \approx 250 \pm 10$ K for DNA, around the same temperatures where the behavior of $< x^2 >$ has a crossover.
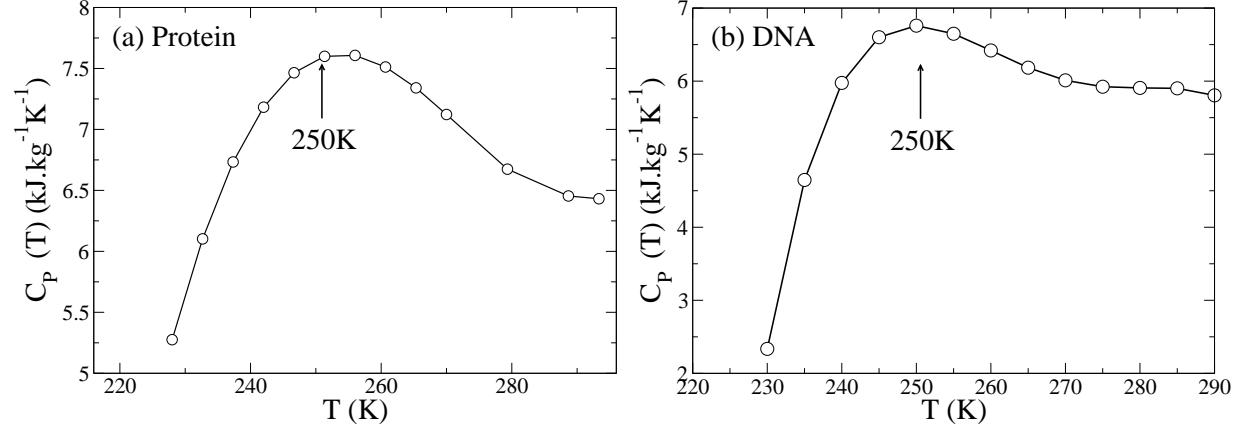
FIG. 2: The specific heat of the combined system (a) lysozyme and water, and (b) DNA and water, display maxima at 250 ± 10 K and 250 ± 12 K respectively, which are coincident within the error bars with the temperature T_{p} where the crossover in the behavior of $< x^2 >$ is observed.
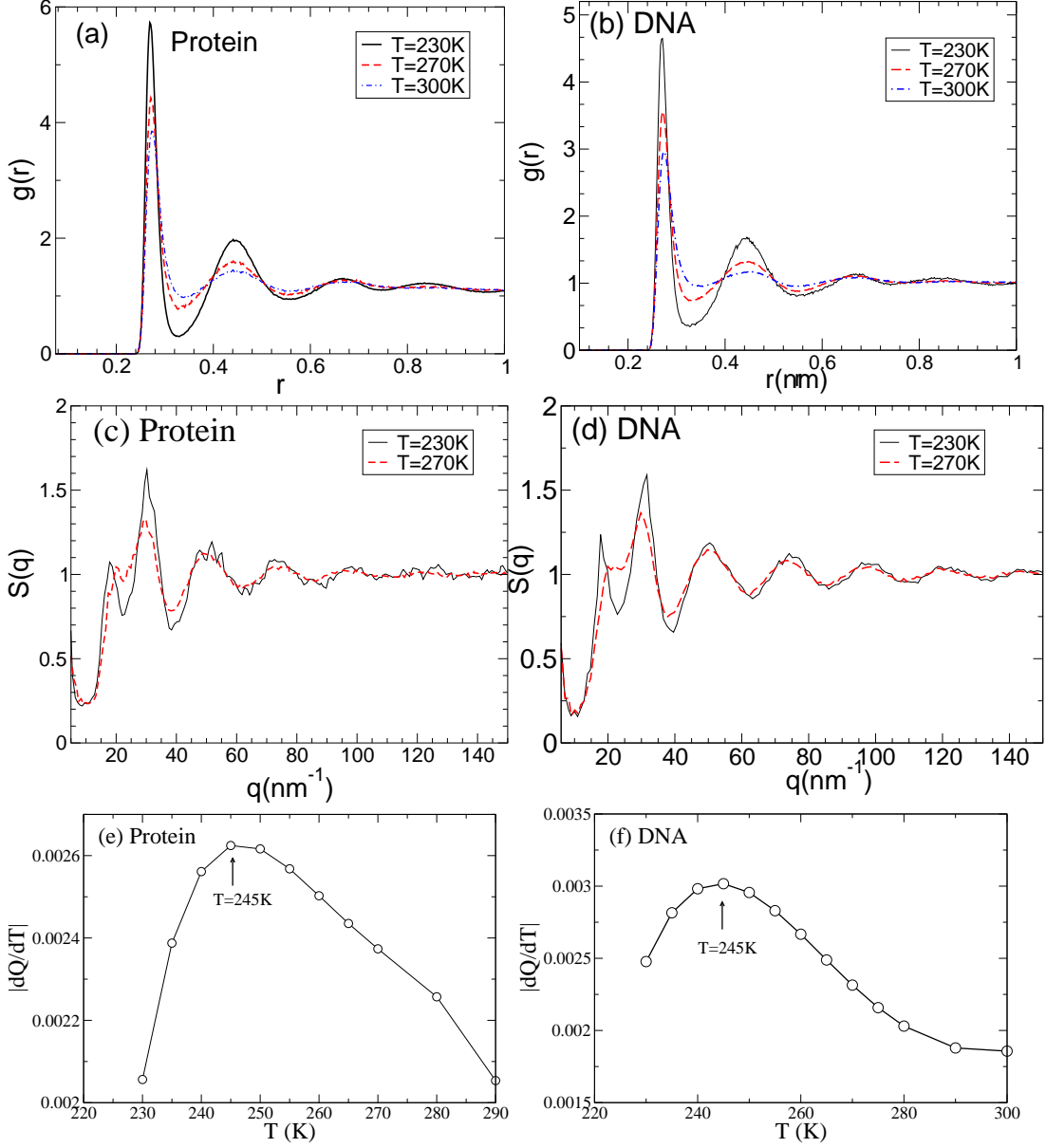
FIG. 3: (Color online) Oxygen-Oxygen pair correlation function $g(r)$ for (a) lysozyme hydration water, and (b) DNA hydration water, on crossing the Widom line from the HDL side ($T = 270$ K, 300 K) to the LDL side ($T = 230$ K). Structure factor of hydration water surrounding (c) lysozyme, and (d) DNA on two sides of the the Widom line. Upon crossing the Widom line, the local structure of water changes from more HDL-like to more LDL-like, reflected in the sharper and more prominent first peak. The first peak associated with the hydrogen bond distance also moves to small wave vectors, suggesting a change from the HDL to the LDL-like local structure of water at low temperatures. Derivative with respect to temperature of the local tetrahedral order parameter Q for (e) lysozyme and (f) DNA hydration water. A maximum in $|dQ/dT|$ at Widom line temperature suggests that the rate of change of local tetrahedrality of hydration water has a maximum at the Widom line.